ATTACHMENT B

B

Amendment

Please Note: The specification as filed contains underlined nucleotide sequences, and as such does not denote a change or amendment. This amendment introduces only Sequence ID numbers. Please amend the specification as follows:

Marked Version

- *On page 21, lines 13 - 31, replace the paragraph in the specification with the following;*

Example 1: Induction of Transcription by Cross-Linking the CD3 Chain of the T-Cell Receptor.

The plasmid pSXNeo/IL2 (IL2-SX) (Fig. 1 of PCT/US94/01617), which contains the placental secreted alkaline phosphatase gene under the control of human IL-2 promoter (-325 to +47; MCB(86) 6, 3042), and related plasmid variants (*i.e.* NFAT-SX, NFB-SX, OAP/Oct1-SX, and AP-1-SX) in which the reporter gene is under the transcriptional control of the minimal IL-2 promoter (-325 to -294 and -72 to +47) combined with synthetic oligomers containing various promoter elements (*i.e.* NFAT, NKB, OAP/Oct-1, and AP1, respectively), were made by three piece ligations of 1) pPL/SEAP (Berger, *et al.*, *Gene* (1988) 66,1) cut with SspI and HindIII; 2) pSV2/Neo (Southern and Berg, *J. Mol. Appl. Genet.* (1982) 1, 332) cut with NdeI, blunted with Klenow, then cut with PvuI; and 3) various promoter-containing plasmids (*i.e.* NFAT-CD8, B-CD8, cx12lacZ-Oct-1, AP1-LUCIF3H, or cx15IL2) (described below) cut with PvuI and HindIII. NFAT-CD8 contains 3 copies of the NFAT-binding site (-286 to -257; *Genes and Dev.* (1990) 4, 1823) and cx12lacZ-Oct contains 4 copies of the OAP/Oct-1/(ARRE-1) binding site (*MCB*, (1988) 8, 1715) from the human IL-2 enhancer; B-CD8 contains 3 copies of the NFB binding site from the murine light chain (*EMBO* (1990) 9, 4425) and AP1-LUCIF3H contains 5 copies of the AP-1 site (5'-TGACTCAGCGC-3', SEQ ID NO 1) from the metallothionen promoter.

- *On page 27, lines 4 - 19, replace the section in the specification with the following;*

5' end of PCR amplified product:

```
          SacII              |----Gal4(1-147)--->>
                             M  K  L  L  S  S  I
5'      CGACACCGCGGCCACCATGAAGCTACTGTCTTCTATCG
                  Kozak
```

3' end of PCR amplified product:

```
      <<----Gal4(1-147----)|
      R  Q  L  T  V  S                (SEQ ID NO 2)
5'    GACAGTTGACTGTATCGGTCGACTGTCG     (SEQ ID NO 3)
3'    CTGTCAACTGACATAGCCAGCTGACAGC
            SalI
```

- *On page 27, lines 30 - 47, replace the section in the specification with the following;*

5' end of PCR amplified product:

```
SacII              |--HNF1(1-281)-->>
                   M  V  S  K  L  S
5'      CGACACCGCGGCCACCATGGTTTCTAAGCTGAGC
          Kozak
```

3' end of PCR amplified product:

```
      <<--------HNF1  (1-282)  ------|
      A  F  R  H  K  L              (SEQ ID NO 4)
5'    CCTTCCGGCACAAGTTGGTCGACTGTCG   (SEQ ID NO 5)
3'    GGAAGGCCGTGTTCAACCAGCTGACAGC
            SalI
```

- *On page 28, lines 11 - 19, replace the section in the specification with the following;*

Insertion of generic start site

```
        Kozak
           _____M  L  E
5'       GGCCACCATGC          (SEQ ID NO 6)
3'    CGCCGGTGGTACGAGCT       (SEQ ID NO 7)

      __          ____
      SacII       XhoI
      overhang    overhang
```

- *On page 28, lines 31 - 41, replace the section in the specification with the following;*

Insertion of NLS into generic start site

```
            T (ACN)
           126                132
      L  D  P  K  K  K  R  K  V  L  E     (SEQ ID NO 8)
5'    TCGACCCTAAGAAGAAGAGAAAGGTAC         (SEQ ID NO 9)
3'         GGGATTCTTCTTCTCTTTCCATGAGCT     (SEQ ID NO 10)

      _____                    _____
      SalI                     XhoI
```

Threonine at position 128 results in a defective NLS.

- *On page 29, lines 14 - 27, replace the section in the specification with the following;*

5' end of PCR amplified product:

```
          SalI |--VP16(413-490)--->>
          _____A P P T D V                  (SEQ ID NO 11)
5'  CGACAGTCGACGCCCCCCCGACCGATGTC           (SEQ ID NO 12)
```

3' end of PCR amplified product:

```
 <<--VP16(413-490)----|
      D  E  Y  G  G                         (SEQ ID NO 13)
5'   GACGAGTACGGTGGGCTCGAGTGTCG            (SEQ ID NO 14)
3'   CTGCTCATGCCACCCGAGCTCACAGC            (SEQ ID NO 15)

           _____
           Xho1
```

- *On page 29, lines 29 - 40, replace the section in the specification with the following;*

Oligonucleotides:

#37 38mer/0.2um/OFF 5'CGACACCGCGGCCACCATGAAGCTACTGTCTTCTA TCG (SEQ ID NO 16)
#38 28mer/0.2um/OFF 5'CGACAGTCGACCGATACAGTCAACTGTC (SEQ ID NO 17)
#39 34mer/0.2um/OFF 5'CGACACCGCGGCCACCATGGTTTCTAAGCTGAGC (SEQ ID NO 18)
#40 28mer/0.2um/OFF 5'CGACAGTCGACCAACTTGTGCCGGAAGG (SEQ ID NO 19)
#43 29mer/0.2um/OFF 5'CGACAGTCGACGCCCCCCCGACCGATGTC (SEQ ID NO 20)
#44 26mer/0.2um/OFF 5'CGACACTCGAGCCCACCGTACTCGTC (SEQ ID NO 21)
#45 26mer/0.2um/OFF 5'GGCCACCATGC (SEQ ID NO 22)
#46 18mer/0.2um/OFF 5'TCGAGCATGGTGGCCGC (SEQ ID NO 23)
#47 27mer/0.2um/OFF 5'TCGACCCTAAGA-(C/A)-GAAGAGAAAGGTAC (SEQ ID NO 24)
#48 27mer/0.2um/OFF 5'TCGAGTACCTTTCTCTTC-(G/T)-TCTTAGGG (SEQ ID NO 25)

- *On page 30, lines 32 - 37, replace the section in the specification with the following;*

The P65 transcription activation sequence contains the following linear sequence:

CTGGGGGCCTTGCTTGGCAACAGCACAGACCCAGCTGTGTTCACAGACCTGGCATCCGTCGACA
ACTCCGAGTTTCAGCAGCTGCTGAACCAGGGCATACCTGTGGCCCCCCACACAACTGAGCCCAT
GCTGATGGAGTACCCTGAGGCTATAACTCGCCTAGTGACAGGGGCCCAGAGGCCCCCCGACCCA
GCTCCTGCTCCACTGGGGGCCCCGGGGCTCCCCAATGGCCTCCTTTCAGGAGATGAAGACTTCT
CCTCCATTGCGGACATGGACTTCTCAGCCCTGCTGAGTCAGATCAGCTCC

(SEQ ID NO 26)

- *On page 31, lines 9 - 19, replace the section in the specification with the following;*

pZHWTx8SVSEAP

A reporter gene construct containing eight tandem copies of a ZFHD1 binding site (Pomerantz *et al.*, 1995) and a gene encoding secreted alkaline phosphatase (SEAP) was prepared by ligating the tandem ZFHD1 binding sites between the Nhe1 and BglII sites of pSEAP-Promoter Vector (Clontech) to form pZHWTx8SVSEAP. The ZHWTx8SEAP reporter contains two copies of the following sequence in tandem:

CTAGCTAATGATGGGCGCTCGAGTAATGATGGGCGGTCGACTAATGATGGGCGCTC GAGTAATGA TGGGCGT (SEQ ID NO 27)

- *On page 32, lines 3 - 21, replace the section in the specification with the following;*

The Xba1 and BamH1 fragment of p65 containing the activation domain was prepared as described above. This fragment was ligated between the Spe1 and BamH1 sites of pCGNN F3.

B. Primers

5'Xba/Zif	**5'ATGCTCTAGAGAACGCCCATATGCTTGCCCT**	(SEQ ID NO 28)
3'Zif+G	**5'ATGCGCGGCCGCCGCCTGTGTGGGTGCGGATGTG**	(SEQ ID NO 29)
5'Not OctHD	**5'ATGCGCGGCCGCAGGAGGAAGAAACGCACCAGC**	(SEQ ID NO 30)
Spe/Bam 3'Oct	**5'GCATGGATCCGATTCAACTAGTGTTGATTCTTTTTTCTTTCTGGCGGCG**	(SEQ ID NO 31)
FKBP 5'Xba	**5'TCAGTCTAGAGGAGTGCAGGTGGAAACCAT**	(SEQ ID NO 32)
FKBP 3' Spe/Bam	**5'TCAGGGATCCTCAATAACTAGTTTCCAGTTTTAGAAGCTC**	(SEQ ID NO 33)
VP16 5' Xba	**5'ACTGTCTAGAGTCAGCCTGGGGGACGAG**	(SEQ ID NO 34)
VP16 3' Spe/Bam	**5'GCATGGATCCGATTCAACTAGTCCCACCGTACTCGTCAATTCC**	(SEQ ID NO 35)
P65 5' Xba	**5'ATGCTCTAGACTGGGGGCCTTGCTTGGCAAC**	(SEQ ID NO 36)

p65 3' Spe/Bam **5'GCATGGATCCGCTCAACTAGTGGAGCTGATCTGACTCAG**

(SEQ ID NO 37)

- *On page 36, lines 15 - 32, replace the section in the specification with the following;*

Construct encoding FRAP domain(s)-VP16 transcriptional activation domain(s)-epitope tag. The starting point for assembling this construct was the eukaryotic expression vector pBJ5/NF1E, described in PCT/US94/01617. pBJ5 is a derivative of pCDL-SR (*MCB* 8, 466-72) in which a polylinker containing 5' SacII and 3' EcoRI sites has been inserted between the 16S splice site and the poly A site. To construct pBJ5/NF1E a cassette was cloned into this polylinker that contained a Kozak sequence and start site, the coding sequence of the SV40 T antigen nuclear localization sequence (NLS), a single FKBP domain, and an epitope tag from the *H. influenza* haemagglutinin protein (HA), flanked by restriction sites as shown below:

```
     Kozak       SV40 NLS                          FKBP(5')
     _____ M  E  D  P  K  K  K  R  K  V  L  E  G  V  Q  V  E ...
CCGCGGCCACCATGCTCGACCCTAAGAAGAAGAGAAAGGTACTCGAGGGCGTGCAGGTGGAG...
SacII         (X/S)                              XhoI

   FKBP(3')_______       HA(flu)tag_________________
.. L  L  K  L  E  V  D  Y  P  Y  D  V  P  D  Y  A  E  D End (SEQ ID NO 39)
..CTTCTAAAACTGGAAGTCGACTATCCGTACGACGTACCAGACTACGCACTCGACTAAGAATTC
              SalI                                    (X/S)    EcoRI
                                                              (SEQ ID NO 38)
```

- *Beginning on page 37, line 32, extending to page 38, line 40, replace the section in the specification with the following;*

5' ends of amplified products:

FRAP fragment a (full-length: primer 1)

```
          L  E  L  G  T  G  P  A  A      (SEQ ID NO 41)
5' CGAGTCTCGAGCTTGGAACCGGACCTGCCGCC      (SEQ ID NO 40)
       XhoI
```

FRAP fragment b (residues 2012-2144: primer 2)

```
          L  E  V  S  E  E  L  I  R      (SEQ ID NO 43)
5' CGAGTCTCGAGGTGAGCGAGGAGCTGATCCGA      (SEQ ID NO 42)
       XhoI
```

FRAP fragment c (residues 2025-2114: primer 3)

```
          L  E  E  M  W  H  E  G  L      (SEQ ID NO 45)
5' CGAGTCTCGAGGAGATGTGGCATGAAGGCCTG      (SEQ ID NO 44)
       XhoI
```

3' ends of amplified products:

FRAP fragment a (full-length: primer 4)

```
    I  G  W  C  P  F  W  V  D            (SEQ ID NO 47)
5' ATTGGCTGGTGCCCTTTCTGGGTCGACCGAGT      (SEQ ID NO 46)
3' TAACCGACCACGGGAAAGACCCAGCTGGCTCA
                          SalI
```

FRAP fragment b (residues 2012-2144: primer 5)

```
    L  A  V  P  G  T  Y  V  D            (SEQ ID NO 49)
5' TTGGCTGTGCCAGGAACATATGTCGACCGAGT      (SEQ ID NO 48)
3' AACCGACACGGTCCTTGTATACAGCTGGCTCA
                          SalI
```

FRAP fragment c (residues 2012-2144: primer 6)

```
    F  R  R  I  S  K  Q  V  D            (SEQ ID NO 51)
5' TTCCGACGAATCTCAAAGCAGGTCGACCGAGT      (SEQ ID NO 50)
3' AAGGCTGCTTAGAGTTTCGTCCAGCTGGCTCA
                          SalI
```

- *On page 39, lines 1 - 14, replace the section in the specification with the following;*

5' end of PCR product:

```
413
```

```
          L  E  A  P  P  T  D  V          (SEQ ID NO 53)
5' CGACACTCGAGGCCCCCCCGACCGATGTC          (SEQ ID NO 52)
       XhoI
```

3' end of PCR product:

```
            490
    D  E  Y  G  G  V  D                   (SEQ ID NO 55)
5' GACGAGTACGGTGGGGTCGACTGTCG             (SEQ ID NO 54)
3' CTGCTCATGCCACCCCAGCTGACAGC
             SalI
```

- *On page 39, lines 29 - 39, replace the section in the specification with the following;*

Oligonucleotides:

1	5' CGAGTCTCGAGCTTGGAACCGGACCTGCCGCC	(SEQ ID NO 56)
2	5' CGAGTCTCGAGGTGAGCGAGGAGCTGATCCGA	(SEQ ID NO 57)
3	5' CGAGTCTCGAGGAGATGTGGCATGAAGGCCTG	(SEQ ID NO 58)
4	5' ACTCGGTCGACCCAGAAAGGGCACCAGCCAAT	(SEQ ID NO 59)
5	5' ACTCGGTCGACATATGTTCCTGGCACAGCCAA	(SEQ ID NO 60)
6	5' ACTCGGTCGACCTGCTTTGAGATTCGTCGGAA	(SEQ ID NO 61)
7	5' CGACACTCGAGGCCCCCCCGACCGATGTC	(SEQ ID NO 62)
8	5' CGACAGTCGACCCCACCGTACTCGTC	(SEQ ID NO 63)

- *On page 40, lines 1 - 20, replace the section in the specification with the following;*

Sequence of representative final construct (NRc1V1E):

```
    Kozak      SV40 NLS                      FRAP(2025-2114)
    _____ M  E  D  P  K  K  K  R  K  V  L  E  E  M  W  H  E ...
CCGCGGCCACCATGCTCGACCCTAAGAAGAAGAGAAAGGTACTCGAGGAGATGTGGCATGAA...
SacII        (X/S)                          XhoI
```

```
   FRAP(2025-2114)        VP16(413-490) ...VP16(413-490)
... R  I  S  K  Q  V  D  A  P  P  T  D     D  E  Y  G  G  V  D
...CGAATCTCAAAGCAGGTCGAGGCCCCCCCGACCGAT...GACGAGTACGGTGGGGTCGAC
                  (S/X)                                   SalI

HA(flu)tag
 Y  P  Y  D  V  P  D  Y  A  E  D End   (SEQ ID NO 64)
TATCCGTACGACGTACCAGACTACGCACTCGACTAAGAATTC    (SEQ ID NO 65)
                       (X/S)    EcoRI
```

For additional details and guidance on materials and methods for regulatable transcription based on rapamycin or analogs thereof, see PCT/US96/09948.

- *On page 42, lines 1 - 16, replace the section in the specification with the following;*

1 GCATCAAGCTTCACAAGACAGACTTGCAAAAGAAGG (SEQ ID NO 66)
2 CCATAGAATTCGTCTATAGAGTCGCCACCCTGATGTC (SEQ ID NO 67)
3 GCATCAAGCTTTTTGGCTTAATTCTCTCGGAAACG (SEQ ID NO 68)
4 CCATAGAATTCAGATTTAAAATTCAAATATTGCAGGCAGGA (SEQ ID NO 69)
5 GCATCAAGCTTATGCACAGCTCAGCACTGCTCTGTTG (SEQ ID NO 70)
6 CCATAGAATTCTCAGAAACGTATCTTCATTGTCATGT (SEQ ID NO 71)
7 GCATCAAGCTTATGAAATATACAAGTTATATCTT (SEQ ID NO 72)
8 CCATAGAATTCTTACTGGGATGCTCTTCGAGCTCGAA (SEQ ID NO 73)
9 GCATCAAGCTTCAGAGTGGACGCACAGTAACATGGG (SEQ ID NO 74)
10 CCATAGAATTCAAGGGAAAGCCAGGCGGCTCTCAGG (SEQ ID NO 75)
11 GCATCAAGCTTATGTGTCCAGCGCGCAGCCTCCTCC (SEQ ID NO 76)
12 CCATAGAATTCTTAGGAAGCATTCAGATAGCTCGTC (SEQ ID NO 77)
13 GCATCGAATTCATGTGTCACCAGCAGTTGGTCATC (SEQ ID NO 78)
14 CCATAATCGATCTAACTGCAGGGCACAGATGCCCAT (SEQ ID NO 79)

Restriction sites used for cloning PCR products are underlined.